PE MICROSCOPIC EXAMINATION FOR SPERMATOZOA

A. SCOPE

A.1 The microscopic identification of spermatozoa is a conclusive test to confirm the presence of semen. This test has been used to identify semen since 1837 when Rattier applied this technique to medico-legal investigations.

Microscopy can be performed under normal bright field conditions; however, the refractive index of the sperm and their relatively thin cell walls do not make them readily visible in many preparations. Phase contrast microscopy and/or staining may be used to increase the contrast of the sperm in the preparation.

Many staining methods have been proposed however, most forensic laboratories have adopted the "Christmas Tree" staining method.

B. QUALITY CONTROL

B.1 Each new lot number of Christmas Tree Stain must be tested with a known positive control and a negative control prior to use with casework samples.

B.2 The results must be documented in the Laboratory Asset Management System (LAM).

B.3 If the used quality control measures do not produce the expected result, the reagents will not be used on evidentiary samples and troubleshooting will be performed. New solutions or materials may be required.

C. SAFETY

C.1 Gloves, a face mask, and a lab coat must be worn. Eye protection (e.g. safety glasses or a face shield) must be worn when removing the coverslip from a prepared slide.

C.2 The appropriate manufacturer's product insert must be read prior to performing this procedure for the first time.

C.3 Distinguish all waste as general, biohazard or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 Christmas Tree Stain Solution A and Solution B

D.1.1 These reagents may be used until depleted; however, they must be discarded on their expiration date. Storage will be according to manufacturer's recommendations.

D.2 A known semen stain is used to test the solutions.

D.3 Permount

D.4 70% ethanol

D.5 Deionized water

D.6 Xylene

E. EQUIPMENT

E.1 Microscope slide with fixed cells from the 'Extraction of Possible Semen Stains' procedure

E.2 Cover slip

E.3 Microscope

E.4 Hot plate @ ~ 60°C

E.5 Forceps, scalpel, scissors

E.6 Freezer

E.7 Glass petri dish

F. PROCEDURES

F.1 Stain cells on a microscope slide by covering cells with Christmas Tree Stain Solution A (Kernechtrot Solution) for 5-10 minutes.

F.2 Wash slide gently with deionized water until Solution A washes off. Ensure no excess water is left on the slide. This can be done by allowing slide to air dry or dry on a hot plate at ~60°C.

F.3 Stain cells on microscope slide by covering cells with Christmas Tree Stain Solution B (Picroindigocarmine Solution) for 10-50 seconds. Rinse slide with 70% ethanol (room temperature), then allow slide to air dry or dry on a hot plate at ~60°C.

F.4 Add Permount and protect with a cover slip. Examine under a compound microscope.

F.5 If cells need to be recovered from the Permount of a recently prepared slide for DNA analysis, xylene may be utilized to remove the coverslip and the cells collected by swabbing.

F.6 If cells need to be recovered from the mounting medium for DNA analysis from previously prepared slides, removing the coverslip may be accomplished using either freezing or soaking in xylene.

F.6.1 Place the slide in an approximately -20°C freezer for 3-5 minutes.

F.6.2 Remove the slide from the freezer and pry the cover slip off using forceps or a scalpel blade.

F.6.3 Rinse the slide with xylene to dissolve the mounting medium.

F.6.4 Rinse the slide with ethanol and collect the cells by swabbing.

F.6.5 Alternatively, crack the corner of the coverslip and soak the slide for several hours until the cover slip can be slid or pried from the slide using forceps or a scalpel blade. Note: this may remove markings from the slide.

F.6.6 Rinse the slide with ethanol and collect the cells by swabbing.

G. INTERPRETATION GUIDELINES

G.1 If observed under phase contrast the heads of the spermatozoa will appear darker in color at the distal ends and lighter in color at the proximal ends (note: proximal end is the point of attachment of the head to the tail while the distal end is the farthest point from the point of attachment)

G.2 If observed under bright field the heads will be clear to light in color at the distal ends and darker in color at the proximal ends (essentially opposite to phase contrast).

G.3 Under both conditions the spermatozoa tails and epithelial cells will appear green.

G.4 Semen is positively identified by observing two intact spermatozoa (head and tail) or two sperm heads; or in the event of observing only one intact spermatozoon or one sperm head, a second analyst must confirm this observation and electronically initial the case notes.

G.5 Evaluate observations and document in case notes; the following scale may be used:

G.5.1 Rare: <1/field (specify count if less than 10)

G.5.2 1+: ~1-2/field

G.5.3 2+: ~3-5/field

G.5.4 3+: ~6-10/field

G.5.5 4+: >10/field

G.6 If the analyst observes <10 total sperm during the initial screening, the entire microscope slide must be examined.

G.7 Depending on the type of sample, if no cells of any type are observed during the microscopic examination, the analyst may need to take a second cutting of the sample to demonstrate that the slide preparation and staining process performed correctly.

G.8 Slides must be labeled and placed into a slide holder; they are typically repackaged with the item. The location must be documented in the notes.

H. REFERENCES

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H.3 Gaensslen, R.E. *Sourcebook in Forensic Serology, Immunology, and Biochemistry,* US Government Printing Office, Washington, D.C., 1983

H.4 Poyntz, F.M. and Martin, P.D., "Comparison of p30 and Acid Phosphatase Levels in Post-coital Vaginal Swabs from Donor and Casework Studies", *Forensic Science International,* 1984, 24: 17 – 25

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